Cooperativity is a phenomenon of universal importance in biological systems and has almost as much variety as it has ubiquity. In virtually all ecosystems the cooperativity of groups, species, and individual organisms is evident. The cooperativity known as “mutualism” is one in which one species provides a haven or a metabolic advantage to another species. On a more microscopic level is the “metabolic cooperativity” in which an enzyme or substrate of one pathway can cooperate with another pathway by providing a component that can act as a substrate or a regulator of that pathway. Delving deeper into the molecular realm we find the type of cooperativity that will be the focus of this review: “allosteric cooperativity” (1, 2). The term “allosteric” has been used to describe a ligand-enzyme interaction, which results in a measurable conformational change in proximal and distal regions of that protein. We will categorize as a subdivision of allosteric cooperativity the phenomenon of $i^3$ cooperativity in which the three following conditions are met: (a) the binding of the ligand induces a conformational change in the protein; (b) the conformational changes are intramolecular in the subunits of a multisubunit enzyme; and (c) the sites are initially essentially identical to each other. This type of cooperativity, which has since been shown in many enzymes, receptors, and ion channels, is of critical importance to both evolution and the field of proteomics because it can serve as a general model for the way in which the networks of interacting enzymes of metabolic pathways are regulated. Because these networks of interactions explain a lot of the factors that control these ensembles of networks, they play a major role in the differences in organisms and the understanding of proteomics.

Cooperativity was originally found by C. Bohr in hemoglobin (3); he observed the sigmoid binding curve of O$_2$ to hemoglobin, which he explained by saying that the binding of the first O$_2$ molecule made it easier for the next O$_2$ to bind and hence could be called “cooperative.” The $\alpha$ and $\beta$ chains of hemoglobin satisfy the “essentially identical” criteria for $i^3$ cooperativity because the binding affinities, sequence homology, and/or structural similarity of the O$_2$ binding sites reveal that they are nearly identical but not quite. After the importance of conformational changes was recognized, two different theories of the cooperative mechanism were postulated. One was the theory of Monod, Wyman, and Changeux (1), herein referred to as the MWC model (and also mentioned as the “symmetry” model, “concerted” model, or the “two-stage” model), and the other was the theory of Koshland, Nemethy, and Filmer (2), herein referred to as the KNF model (and often mentioned as the “induced fit” model or the “sequential” model). The MWC model postulated that the subunits changed shape in a concerted manner to preserve the symmetry of the entire molecule as it was transformed from one conformation (T) to a second conformation (R) under the influence of ligand. The alternative KNF model postulated that each subunit changed shape as ligand bound, so that changes in one subunit led to distortions in the shape and/or interactions of other subunits of the protein. A mathematical examination of these theories showed that both gave sigmoid curves and could explain, within experimental error, how O$_2$ bound to hemoglobin. Both theories were postulated to apply to many other enzymes that also gave sigmoid curves showing cooperativity. The KNF model, however, also predicted that in some cases the first ligand to bind could make it more difficult for subsequent ligands to bind. This was called “negative cooperativity” because there was (a) “cooperativity” between the subunits and (b) “negative” because binding of one ligand made the binding of subsequent ligands more difficult (4, 5). The MWC theory allowed no such alternative. Because only the KNF theory fit negatively cooperative enzymes, it is easy to select that model for such enzymes, but because both theories fit positively cooperative enzymes more sophisticated tools must be applied to such cases. However, positive cooperativity and negative cooperativity are easy to distinguish from each other and they are important to proteomics and evolution so we will address their significance first and the mechanism for achieving them next.

To obtain an objective appraisal of the relative quantities of negatively and positively cooperative enzymes in nature, we first selected all publications that had cooperativity in their titles in the period 1980–1990. Tables 1–3 (see supplemental material) are a distillation from 7,316,070 documents in the Science Citation Index from the years 1980–1990 inclusive. Of these, 374 articles had “cooperativity” in the title. From there, articles focusing on enzymes consistent with $i^3$ cooperativity were identified and are listed in Tables 1–3. Table 1 shows 29 of the 291 examples of protein cooperativity reported in the 1980–1990 period (Refs. 19–47). Table 2 shows 27 of the 215 examples of negative cooperativity reported in 1980–1990 (Refs. 48–75), and Table 3 shows 4 of the 61 examples of enzymes that show both negative and positive cooperativity in 1980–1990 (Refs. 76–79).

As can be seen in the list of enzymes given in Tables 1 and 2, the number of negatively cooperative enzymes is approximately the same as the number of positively cooperative enzymes suggesting that the sensitivity capabilities listed above have about equal evolutionary value with a slight evolutionary advantage to positive cooperativity. To check these results, in Tables 4–6 in the supplement are positive cooperativity and negative cooperativity in the years 1991–1993 (Refs. 80–104). Because the relative ratios of enzymes and other proteins with positive and negative cooperativity are roughly the same in the two arbitrarily selected time periods, we can assume that they are probably a pretty good indication of the relative ratios for all enzymes, i.e. about 50% positive and 50% negative. To some this may seem unusual because positive cooperativity was discussed first in relation to hemoglobin and to many people
seems to be the reason that cooperativity exists. The large number of negatively cooperative enzymes shows that this type of cooperativity is not an aberration or a minor curiosity but rather that there must be good evolutionary reasons in metabolic systems for it as well as positive cooperativity. It is of interest, therefore, to consider why each should be selected over evolutionary time (6).

As seen in Fig. 1, positive cooperativity confers the metabolic advantage of amplifying the sensitivity of a signal, i.e., a small change in ligand $L$ can have a far greater effect on the output response in a positively cooperative system than in a Michaelis-Menten system. For example, a 3-fold increase in the ligand ($L = O_2$) concentration for hemoglobin changes the binding capacity 9-fold (from 10 to 90%), whereas in a Michaelis-Menten system, it requires an 81-fold change in ligand concentration to go from 10% binding capacity to 90% binding capacity. It is important, for example, that hemoglobin picks up the maximum amount of $O_2$ in the lungs and unloads the maximum amount of $O_2$ to the tissues, a biological phenomenon for which positive cooperativity is very important (7, 8). In fact, humans with hemoglobin mutations that lack the cooperativity are very sick people (9). Negative cooperativity, on the other hand, decreases the sensitivity to Michaelis-Menten, but in the process of doing that, it extends the range over which some response is generated. CTP acts as an allosteric inhibitor to inhibit carbamoyl-phosphate synthetase, which is at a branch point that leads to several other products besides CTP (10). Therefore, it is important that excess CTP not shut down the carbamoyl-phosphate synthetase enzyme completely because all of the other pathways would be inhibited as a consequence, so negative cooperativity is observed for CTP binding to carbamoyl-phosphate synthetase, which makes it very difficult to shut down the enzyme completely even with great excess of CTP. Although the present review does not prove that cooperativity is correlated with being a branch point enzyme, there seems to be a general trend indicating such a correlation.

A second reason for the importance of $i^o$ cooperativity may be its ability to shift the range of sensitivity without changing the amino acid sequences of the active site. In fact it is observed that there is a different midpoint of the affinity curve for $O_2$ between frogs and tadpoles (11). One way that might have been tried to achieve such a change would be to alter amino acids where $O_2$ binds to hemoglobin, but that binding site is exquisitely designed so that the ferrous ion of the heme is prevented from getting oxidized to ferric ion (7–9). The adjustments in side chain amino acids and their precise geometry have been selected over evolutionary time, and it is not clear that a new active site could be designed that was as effective at a different

![Fig. 1](image1.png)

**Fig. 1.** The binding curve of a ligand to a protein with four identical subunits, each of which has one site for binding of a ligand ($L$). $a$, curve with positive cooperativity; $b$, curve with no cooperativity (Michaelis-Menten); and $c$, curve with negative cooperativity. Relative stimulus is stimulus, $S$, divided by stimulus when protein half-saturated, $S_{0.5}$.

![Fig. 2](image2.png)

**Fig. 2.** How the midpoint of a binding curve can change by several orders of magnitudes by changing only the subunit interactions ($K_{AB}$ and $K_{AB}$). Curves are shown in which $K_{AB}$ and $K_{AB}$ are changed in factors of 10 with all other interactions such as $K_{AA}$ and $K_{BB}$ and $K_{AB}$ remaining constant. The midpoints vary from $10^{-2}$ to $10^{-4}$ for curves of equal steepness. (Examples are from a 4-subunit protein using the KNF model.)
O₂ concentration range, so the same active site is used for both tadpole and frog but the intersubunit contacts, which are far from the active site, are mutated. How the subunit contacts change Sα,β is the midpoint of the curve without changing the cooperativity (steepness) in the KNF model is shown in Fig. 2, where a number of curves of the same steepness are seen to have different midpoints (2) because they have different subunit interrelations. This property is used to optimize the O₂ concentration range from aqueous ponds to dry land by changing subunit contacts without tampering with the active site (11). This ability to change the midpoint of a saturation curve without changing its active site has undoubtedly been useful in changing the responsiveness range of the same enzyme in different tissues or in different species.

At this point it may be useful to examine, first, whether it is appropriate to retain simplified models when x-ray crystallographic pictures exist, and second, which model, the concerted or the sequential, is more likely to be correct. First, the simplification with circles and squares for conformations is valuable even in the age of crystallography. By analogy, Schrodinger’s equation is a far more accurate representation of the electrons and orbits of an atom than a simplistic symbol like H⁺ or Cu²⁺, but it will be a long time before a chemist will find the Schrodinger equation more useful in describing a simple reaction than using a simplification like Cu²⁺ + Fe²⁺ = Cu⁺ + Fe³⁺, so the simple models explain the phenomenon and the crystallography and complex math give the accurate details.

Thus in the case of hemoglobin, Holt et al. (8) using spin labels and NMR have shown clear examples of a stepwise change in structure that is in agreement with the KNF model but also shows a switch between two quaternary structures, which is in agreement with the MWC model. Perutz, an initial advocate of the MWC model for Hb, has recently said (12) the changes induced by oxygen indicated that both the MWC model and the KNF model are partly right, and Holt and Ackers (13) have proposed a sequential model with a switch in quaternary structure at the 2O₂ bound state. In the case of aspartyl transcarbamoylase, Stevens and Lipscoum (14) showed a combination of sequential intrasubunit conformational changes on a broad background of a quaternary change.

In the second place the KNF model can explain all cases, i.e. positive, negative and no cooperativity; where there is a ligand-induced conformational change, it is logical to deduce that KNF is the general model and see whether there are circumstances in which it would reduce to the MWC model. In fact, a calculation to clarify the relationship of the models to the protein forces in an enzyme was made (15) and shows that the general KNF model reduces to the MWC model or the simplified KNF model when appropriate subunit parameters are chosen (14). Moreover, the KNF model can explain cases like aldolase and lactic dehydrogenase, where there are big changes in the conformation and no cooperativity by assuming KAB = KBB, i.e. no change in subunit interactions but still a change in the conformation of the individual subunits. Another class of enzymes that must fit the KNF model is those that show both positive (with one ligand) and negative cooperativity (with another ligand) or those that show both positive and negative cooperativity with a single ligand during the sequential binding of that ligand.

Thus, a general way of looking at cooperativity is to say the ligand usually induces a sequential change such that the conformation change caused by the first ligand is transmitted to neighboring subunits in such a way that it may make subsequent ligands bind (a) more easily, (b) less easily, or (c) without any effect. In one extreme, the binding of the first molecule can cause all the subunits to change in a concerted way so that symmetry is preserved, and at another extreme they can change the conformation sequentially subunit by subunit as each ligand is bound with no net effect on neighboring subunits. The sequential binding of ligand may at some point cause the protein to shift from one quaternary structure to another (say T to R or A₁ to B₁). An elegant use of physical tools to distinguish between the models for positively cooperative enzymes has been developed by Ho and co-workers (17).

It is intriguing in this connection that it was recently found that an artificial point mutation changes pyruvate kinases from no cooperativity to positive cooperativity (16). Similarly, the aspartyl receptor can be changed from positive to negative or to no cooperativity by changes in a single amino acid, Ser-68, in the subunit interface of that enzyme (17). Thus, cooperativity in a multisubunit protein depends on a delicate balance between many forces in which it seems likely that a small change in ligand or an amino acid mutation can change the cooperativity and allosteric properties of an enzyme, so it becomes apparent how easy it would be in evolution to get the appropriate cooperativity. The initial pattern becomes solidified by selection to favor a cooperativity that benefits that organism, so allosteric cooperativity has been preserved and improved over evolutionary time as a mechanism that can increase sensitivity, expand the range, and adapt metabolism to a change in conditions such as the change in conditions from sea to land. Cooperativity is one of the established phenomena that makes living possible and will take its place as a key factor in the proteomics of the cell.

REFERENCES
Proteomics and Models for Enzyme Cooperativity
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